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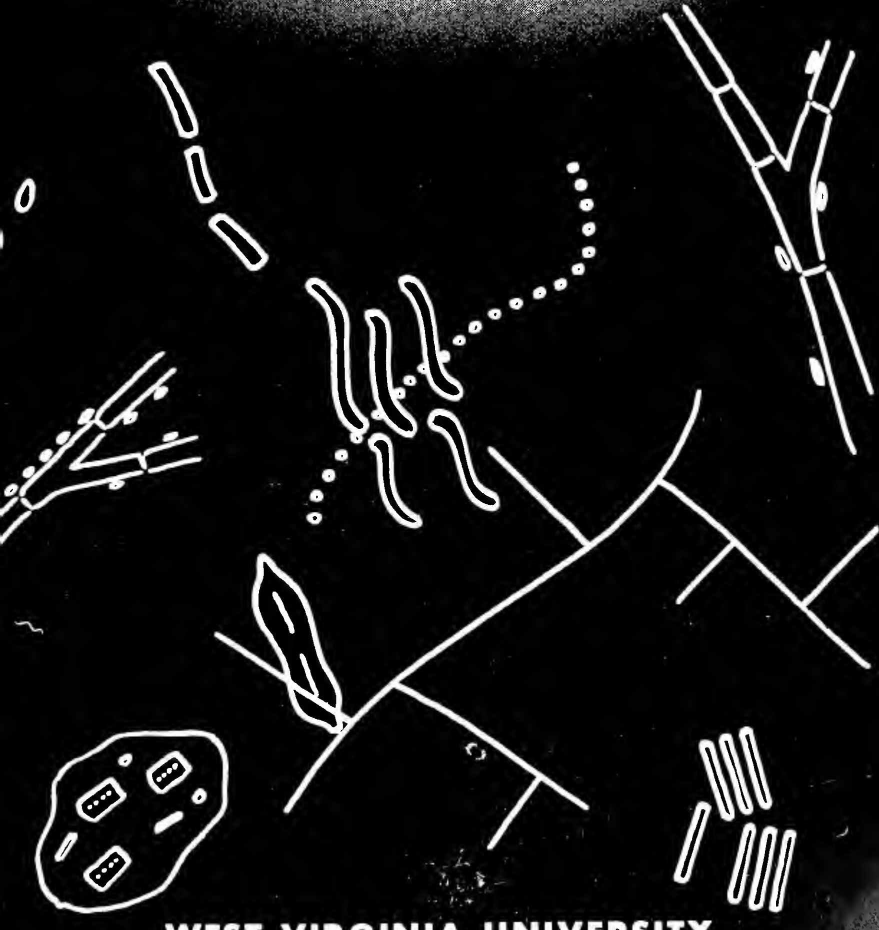
THE MICROBIOLOGY OF STRIP-MINE SPOIL



WEST VIRGINIA UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

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The MICROBIOLOGY Of Strip-Mine Spoil

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The Microbiology Of Strip-Mine Spoil

Introduction

H. A. WILSON

THE OVERBURDEN above a coal seam that is piled aside during the strip mining of coal is known as spoil. The various strata are not kept separate but are intermixed. In general, the original top layers become the bottom layers of the spoil and those closer to the coal seam become the spoil surface layers. Where the topography is more or less level the spoil is piled in ridges that are roughly parallel (Figure 1). In hillside stripping operations it is pushed aside. Spoil, regardless of the type of area stripped, forms an unsightly scar upon the landscape.

Newly formed spoils are bare of vegetation and their nitrogen and organic content is almost nil. Many such spoils, however, if fertilized and seeded will support vegetation. If not fertilized and seeded they will remain barren, often for many years, and only slowly will they become naturally reseeded to a few herbaceous weeds and such grasses as poverty grass (*Danthonia spicata* [L.] Beauv.), broomsedge (*Andropogon* sp.), and deertongue (*Panicum clandestinum* L.).

Within a few years after formation some spoils will become strongly acid. Such spoils result from the strip mining of coal seams that have pyritic materials associated with them. These pyritic materials in the unstripped strata are in a reduced environment, but when exposed at or near the surface they are in an oxidative environment. In this latter environment the reduced sulfur becomes oxidized to sulfuric acid through microbiological action. The chemical changes involved have been discussed by Colmer and Hinkle (4).

Although the management of spoil areas has received much study, considerable controversy prevails concerning the socio-economic aspects not only of strip mining but of the spoil itself. Problems of conservation, revegetation, the effect spoils have upon streams within the area, aesthetic problems, and problems arising from land use and the effect upon adjacent farmsteads are all involved in the overall management of spoil (11).

Publications dealing with the revegetation of spoil areas are voluminous. Such studies report both successes and failures in revegetation of such areas. The major problems arise from the acidic spoils, which often have pH levels well below the minimum tolerated by most plant life.

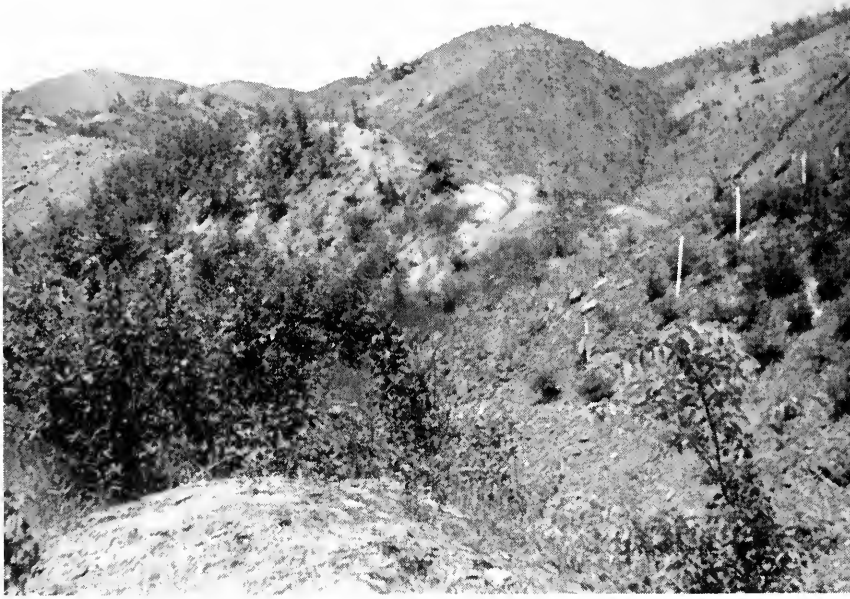

FIGURE 1. Spoil in ridges—strip mining on level topography (Indiana).

Once vegetation is established on a spoil its continued success depends upon the physical, chemical, and biological factors that are so complexly interrelated in the soil (spoil) condition known as fertility. This publication is based upon a collection of studies made by the author and some of his co-workers on the microbiology of spoil. Most of the results of the studies have been published previously. All questions relating to the microbiology of spoil have not been answered but it is hoped that the data will contribute toward the knowledge necessary for efficient management of strip-mine spoils.

Strip-Mine Spoil Areas

In all studies on strip-mine spoil, the samples from each area were obtained, when available, from a nonvegetated or barren spoil, a vegetated spoil, and an undisturbed area adjacent to the spoil area but not directly influenced by the stripping operations.

All samples were composite samples, each consisting of three or more sub-samples representing the 0 to 4- or 0 to 5-inch depths. In some studies, 4 to 8-inch depth samples were also obtained. All samples were passed through a ⅛- or ¼-inch mesh hardware cloth in order to remove stones, bits of coal, and plant roots.

Most of the samples were obtained from strip-mine areas in Monongalia and surrounding counties, or in Pennsylvania near the West Virginia state line. The iron ore samples were obtained in Preston County. Listed below are the areas and the available information concerning them. The names in most instances were given by the author for identification purposes only.

CANYON: Monongalia County. This area was stripped in 1943 and the spoil was leveled. Tyner and associates (24, 25) limed, fertilized, and seeded a portion of the area to grasses and legumes.

DIXON: Monongalia County. Naturally reseeded to *Andropogon* sp.

ARTHURDALE: Preston County. The stripping operations probably were made in 1943-44 or earlier. One small section of the area was limed, fertilized, and seeded to forage plants by Tyner *et al.* (24, 25). Other portions of the area were planted in 1945 to pine (*Pinus* sp.) and black locust (*Robinia pseudo-acacia* L.) by the Division of Forestry, West Virginia University. Neither lime nor fertilizer was used in the pine-locust planting.

CHEAT: Preston County. This area was on a ridge top and portions of it had become naturally reseeded to poverty grass (*Danthonia spicata* [L.] Beauv.) and broomsedge (*Andropogon glomeratus* [Walt] BSP).

BRUCETON: Preston County. This area had been limed, fertilized, and reseeded to alsike clover (*Trifolium hybridum* L.), red clover (*Trifolium pratense* L.), and alfalfa (*Medicago sativa* L.).

FAIRMONT: Marion County. Nothing is known concerning this area except that it was covered with deertongue (*Panicum clandestinum* L.) and an unidentified bunch grass through natural reseeding.

KAUFMANN: Marion County. The leveling of this small area was completed in 1947 after which it was seeded to lespedeza (*Lespedeza stipulacea* Maxim.). No other information was available.

PRUNTYTOWN: Taylor County. This mining operation was on the Pruntytown Industrial School Farm. Stripping operations were finished in 1946; the area was leveled in 1947 and a portion was fertilized and seeded to soybeans. The following year wheat was planted as a companion crop for a grass-clover seeding.

POINT MARION: Fayette County, Pennsylvania. No information was available. One portion of the spoil apparently had been reseeded and when sampled was an overgrazed pasture of Kentucky bluegrass (*Poa pratensis* L.) and white Dutch clover (*Trifolium repens* L.). The vegetation of the adjacent undisturbed soil was mostly Kentucky bluegrass and *Andropogon* sp.

West Virginia-Pennsylvania (WV-Pa): Fayette County, Pennsylvania. Some attempt possibly had been made to reseed the strip-mine spoil as both alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) were present. The pH of the soil was also indicative of this.

Pennsylvania: Fayette County. An abandoned area which had become naturally reseeded to *Andropogon* sp.

The iron ore spoil areas were formed sometime between 1822 and 1866. At the time these were sampled they were covered by stands of oak (*Quercus* sp.) and yellowpoplar (*Liriodendron tulipifera* L.) about 30 years old (22). An A_1 horizon had developed in these spoils. The three areas are designated as Johnson Hollow, Quarry Run, and Cheat. All were in Preston County, West Virginia.

Materials and Methods

All pH determinations were made electrolytically on a soil (spoil): water 1.5: 1 mixture which had stood for one hour with frequent shaking. The moisture determinations were made by drying the spoil and soil to constant weight at 105-110° C.

Further details concerning materials and methods will be described in connection with the experiments discussed or reference will be made to the original publications for the details.

Studies on Microorganisms

NUMBERS OF BACTERIA, FUNGI, AND ACTINOMYCETES (33)

The numbers of microorganisms in spoil are influenced by a number of environmental factors, including pH, energy sources, nitrogen and other elements, moisture, and temperature.

Since organic matter in spoil is essentially nil, nitrogen also is limited. Many spoils, particularly in the West Virginia coal region, become highly acid; pH levels between 3.0 and 3.5 are not uncommon. At times, particularly during the summer, the levels of both moisture and temperature are unfavorable for microbial growth, especially at the spoil surface.

Methods[1]

Nonvegetated (barren), vegetated (reseeded by man or naturally reseeded), and undisturbed (adjacent soil) samples were analyzed. The samples represented the 0 to 4- and 4 to 8-inch depths. All samples were

[1]Complete details are given in: H. A. Wilson and Gwendolyn Stewart, *The Number of Bacteria, Fungi, and Actinomycetes in Some Strip-Mine Spoil*, W. Va. Agr. Expt. Sta. Bull. 388T, Feb., 1956, 15 pp.

sieved at the site of collection, brought to the laboratory, and placed in a refrigerator until plated. All platings were made the same day that the samples were collected.

Standard plating procedures were used to determine the numbers of bacteria, fungi, and actinomycetes. Sodium caseinate agar was used to determine the numbers of bacteria and a glycerol agar for the actinomycetes (10). Rose bengal agar (20) was the plating medium for the fungi. Incubation was at 25 ± 1° C for 3 days for the fungi, 5 days for the bacteria, and 10 days for the actinomycetes.

Results

The number of bacteria, fungi, and actinomycetes determined in nonvegetated and vegetated spoil and in adjacent undisturbed soil, as well as the pH of the materials, are presented in Table 1.

Bacteria. The numbers of bacteria were low in the nonvegetated spoil. Instead of a 1 to 5 million bacterial density per gram, as often encountered in an arable soil, as determined by the standard plate count, only 6 to 101 thousand were found.

The bacterial density of the vegetated spoil, on the other hand, was as high as 1 million or more per gram. In some instances, the numbers were even larger than those found in an adjacent undisturbed soil.

In general, more bacteria were found in the 0 to 4-inch layer of spoil than in the 4 to 8-inch layer.

Table 1. Numbers (in Thousands) of Bacteria, Fungi, and Actinomycetes Per Gram of Strip-Mine Spoil (Oven-Dry Basis)

Spoil Areas and Types	Microorganism						Soil Acidity (pH)	
	Bacteria		Fungi		Actinomycetes			
	0-4*	4-8	0-4	4-8	0-4	4-8	0-4	4-8
Canyon								
Nonvegetated	6	26	8	10	1	5	3.28	3.40
Vegetated	1135	141	280	35	181	23	3.88	3.35
Undisturbed ..	1354	805	49	23	1980	444	4.70	4.88
Pruntytown								
Nonvegetated	101	61	37	30	19	38	4.02	4.82
Vegetated	3050	111	360	26	220	154	3.78	3.14
Undisturbed ..	4630	1920	395	59	4360	3270	6.52	6.78
Kaufmann								
Nonvegetated	12	38	133	29	140	9	3.46	3.70
Vegetated	21120	2274	181	39	4500	21360	6.95	7.00
Undisturbed .	5250	2220	252	56	2260	1130	5.22	4.98
Arthurdale								
Nonvegetated	41	41	17	17	6	8	3.75	3.75
Vegetated	5840	3390	51	32	2410	367	6.00	5.30
Pines	583	281	79	25	274	121	4.72	4.62
Locusts	1120	357	62	3	147	18	4.49	4.52
Undisturbed ..	11210	1296	213	32	2420	1520	5.30	5.70

*Depth in inches.

More bacteria were found in spoil from the black locust planting than in that from the pine planting. The numbers, however, were not equal to those found in spoil supporting a vegetative cover of forage grasses and legumes.

Fungi. The nonvegetated spoil averaged fewer fungi per gram than did the vegetated spoil and more fungi were generally found in the 0 to 4-inch layer of spoil than in the 4 to 8-inch layer. Although there is considerable question as to the value of fungal counts, in no spoil area did the total number of fungi in the nonvegetated spoil approach the number in the undisturbed soil and in only one instance exceeded the numbers in vegetated spoil.

The spoil from the pine planting contained more fungi per gram than that from the black locust planting. This is the reverse of results found with bacteria.

No definite trend appears when the fungal counts of vegetated spoil are compared to undisturbed soil. In some instances more fungi were found in the vegetated spoil and at other times the undisturbed soil contained the larger number.

Actinomycetes. In all samples the number of actinomycetes in nonvegetated spoil was less than the number in vegetated spoil. Except for the Kaufmann area samples, more actinomycetes were found in the undisturbed soil than in vegetated spoil.

The spoil from the pine planting apparently supported a greater actinomycete population than did the spoil from the black locust planting. This was the same as for the fungi and the reverse for the bacteria.

Discussion

These data definitely show the influence of vegetation upon the numbers of bacteria, fungi, and actinomycetes in strip-mine spoil, even though the effect of lime applied at the time of seeding a spoil may have disappeared, and the spoil is again moderately to strongly acid. Vegetated spoil shows certain characteristics similar to unstripped soil in regard to microbial population. Larger numbers of organisms were usually found in the 0 to 4-inch layer of vegetated spoil and undisturbed soil than in the 4 to 8-inch layer. In some nonvegetated spoil more microorganisms were present in the 4 to 8-inch depth samples.

Although nonvegetated acid spoil contains some microorganisms, a similar spoil of the same pH, but vegetated, will contain a much larger microbial flora. In many instances the bacterial density of a vegetated spoil, for example, was approximately the same as the microbial density of an adjacent unstripped soil.

These data indicate that the vegetation exerts a greater influence upon the microflora of a spoil than does the pH, but one must not forget that the vegetation is influenced by the pH.

NUMBERS OF RHIZOSPHERE BACTERIA (28)

For some years the interrelationships among rhizosphere organisms and plant roots growing in soil have been under investigation by many workers. A study was made to determine whether the numbers of bacteria in the rhizosphere of plants growing on spoil materially differed from the numbers outside the root influence.

Methods[2]

The Bruceton and Pennsylvania areas were used in this study. Spoil samples from the roots of plants were obtained as follows: The roots of isolated plants were loosened with a spade and gently lifted from the spoil. The plant was then gently shaken to free the roots of loosely adhering spoil. It then was again gently shaken, this time over sterile kraft paper. This fraction of spoil was designated as "rhizosphere." The roots were separated from the shoots and placed in sterile jars.

Dilutions for use in determining the total bacterial counts of roots by the standard plate count method were as follows: One gram of roots was added to a 100-ml sterile water blank and permitted to soak for five minutes, after which the bottle was gently shaken for one minute. All root pieces were then removed and placed in another water blank containing 10 grams of sterile sand. The bottle from which the roots had been removed contained only sediment and is designated as "rhizoplane." After serial dilutions were made and plated from the sediment bottle, determinations were made in order to calculate the sediment weight.

Total bacterial counts were made using soil extract isolation medium as a plating medium (15). Plates were incubated at 25° C for seven days. Some bacteria were picked from the plates for future study.

Results

The data are presented in Table 2. In this, as in other studies, the total number of bacteria in nonvegetated spoil was low. The Bruceton nonvegetated spoil, with a pH of 3.0, contained only 8 thousand bacteria per gram (oven-dry basis). The Pennsylvania spoil contained 70 thousand, but it had a pH of 4.3.

[2]Details of this experiment were reported in: H. A. Wilson, "Rhizosphere Bacteria of Some Strip-Mine Vegetation," *Proc. W. Va. Acad. Sci.* 33: 15-20. 1961.

TABLE 2. TOTAL COUNTS OF BACTERIA. COUNTS x 10^6

Spoil	Roots	Rhizoplane	Rhizosphere	pH*
PENNSYLVANIA				
Nonvegetated			.07†	4.3
Andropogon	2.9	74.1	.36	4.7
BRUCETON				
Nonvegetated			.008†	3.0
Alsike	1,150.0	62.7	26.8	3.5
Red Clover	208.0	7.4	1.2	5.5
Alfalfa	47.2	188,870.0	.47	5.8

*These values refer only to the nonvegetated and rhizosphere samples.

†To conserve tabular space the counts for the nonvegetated samples were placed under "Rhizosphere."

The "Andropogon" rhizosphere bacteria numbered only 360 thousand as compared to 74.1 million in the rhizoplane and 2.9 million on the roots.

The counts from the Bruceton samples were somewhat erratic, except that the number of bacteria in the rhizosphere of each plant type was less than in the rhizoplane and on the roots. The alsike clover and red clover roots showed bacterial numbers far greater than those associated with either their rhizoplane or their rhizosphere. In all instances the number of bacteria found in the rhizosphere was less than the number found in the rhizoplane or on an equal weight of roots.

Discussion

Greater numbers of bacteria were obtained from the plant roots, rhizoplane, and the rhizosphere of vegetated spoil than from the nonvegetated spoil. Greater numbers of bacteria were obtained from the surface of an equal weight of roots and rhizoplane than from the material designated as rhizosphere. This is probably due to the better energy supply for the bacteria and also a more favorable pH environment close to the roots.

IDENTIFICATION OF FUNGI (33)

Filamentous fungi were present in all spoil samples plated for microbial counts. The presence of vegetation influenced the density of these microorganisms in the same way that it influenced the density of bacteria and actinomycetes. From two spoil areas, Arthurdale and Canyon, samples were plated and all fungi which appeared on the plates were identified.

The fungal isolations were made on Rose bengal agar (20) from seven different samples. Twenty-two isolates were identified to genus. The genera and the type of spoil from which they were isolated are presented in Table 3.

TABLE 3. GENERA OF FUNGI FOUND IN TWO STRIP-MINE SPOIL AREAS†

Fungi	Spoil Areas								Total
	Canyon			Arthurdale					
	Non-Vegetated	Vegetated	Undisturbed	Non-Vegetated	Vegetated	Pine	Locust	Undisturbed	
Acromoniella			*						1
Alternaria				*					1
Aspergillus	*	*		*	*	*	*	*	7
Beauveria			*					*	2
Chalara						*			1
Cladosporium	*		*						2
Cunninghamella	*	*	*	*	*	*	*		7
Epicoccum	*								1
Fusarium			*		*				2
Gliocladium	*		*		*				3
Helminthosporium		*	*					*	3
Isaria							*		1
Melanospora								*	1
Mucor		*							1
Myrothecium				*	*				2
Penicillium	*			*		*	*		4
Spicaria							*	*	2
Stemphilium				*					1
Stilbella					*				1
Trichoderma	*	*	*	*	*	*	*	*	8
Verticillium			*		*				2
Zygorrhynchus			*	*	*				3

†The author wishes to thank Dr. H. L. Barnett for the identification of the fungi.
*Denotes presence; no marking denotes the fungus was not found.

Discussion

The variety of fungi identified indicates that a fungal population is found in the nonvegetated spoil as well as in vegetated spoil. Unfortunately, the procedure used in this study failed to indicate which, if any fungi, were in a vegetative condition or present in the spoil simply as spores. Some, however, must be in a vegetative stage since hyphal fragments were found on slides buried in spoil (see Qualitative Observations below). Although these data are meager, they do indicate that vegetation on spoil, to some extent, influences the fungi that are present.

QUALITATIVE OBSERVATIONS (30)

The Rossi-Cholodny buried slide technique (3, 19) is a well-known soil microbiological procedure. It is qualitative in nature and the isolation of microorganisms is practically impossible. Yet as a supplemental procedure to other techniques, it yields valuable information of qualitative changes among the microorganisms resulting from additions to the soil.

Methods[3]

Spoil representing the 0 to 5-inch depth from the Point Marion area was used in this study. One hundred-fifty grams (oven-dried basis) of the spoil were placed in straight-sided water tumblers and then 0.75 gm autoclaved ground oat straw or ground alfalfa was added and the contents thoroughly mixed. Two 3 x 1-inch clean and sterile microscope slides were inserted into each tumbler, and an amount of water, previously calculated, was added to bring the moisture of each tumbler's contents to 50 per cent of its water-holding capacity. The tumblers were incubated at 25° C for 7 and 14 days. During incubation water was added to replace any lost by evaporation.

One slide of each pair was carefully removed after 7 days and the other after 14 days of incubation. Each slide was air dried, fixed over a flame and then heat stained for 5 minutes with Rose bengal, after which it was examined microscopically. Some composite microscopic fields drawn from actual observations are shown in Figure 2.

Results and Discussion

(Nonvegetated Spoil.) The bacteria on slides buried in nonvegetated spoil were few and scattered. Although some long slender rod-shaped bacteria were observed, the predominant forms were rather large plump rods, often occurring in pairs. More fungi and actinomycetes were observed on slides buried for 14 days than for 7 days. The addition of straw and alfalfa to the spoil resulted in an increase in numbers as well as types of organisms; the addition of alfalfa caused the largest increase.

(Vegetated Spoil.) On slides buried in vegetated spoil, with neither straw nor alfalfa added (control), more bacteria, fungi, and actinomycetes were observed than on those slides that had been buried in nonvegetated spoil with alfalfa or straw. More spore-forming rods were observed in the vegetated control spoil than in the nonvegetated + alfalfa, but slender rods predominated. The addition of straw seemed to favor the development of medium-sized rods. Some spherical forms also were observed. The straw favored an increase in fungal hyphae over the control. Spherical, short plump and long slender rods were numerous on slides that had been buried in the spoil to which alfalfa had been added. The slides also contained many fungal and actinomycete hyphae.

(Undisturbed Soil.) The addition of ground straw or alfalfa to the soil did not result in as much of a microbial change as when these materials were added to the spoil. Coccobacilli and medium-sized rods seemed to predominate in the control. The most noticeable difference

[3]Details were given in: H. A. Wilson and H. G. Hedrick, "Some Qualitative Observations of the Microflora in a Strip-Mine Spoil," *Proc. W. Va. Acad. Sci.* 29-30: 35-38. 1957-58.

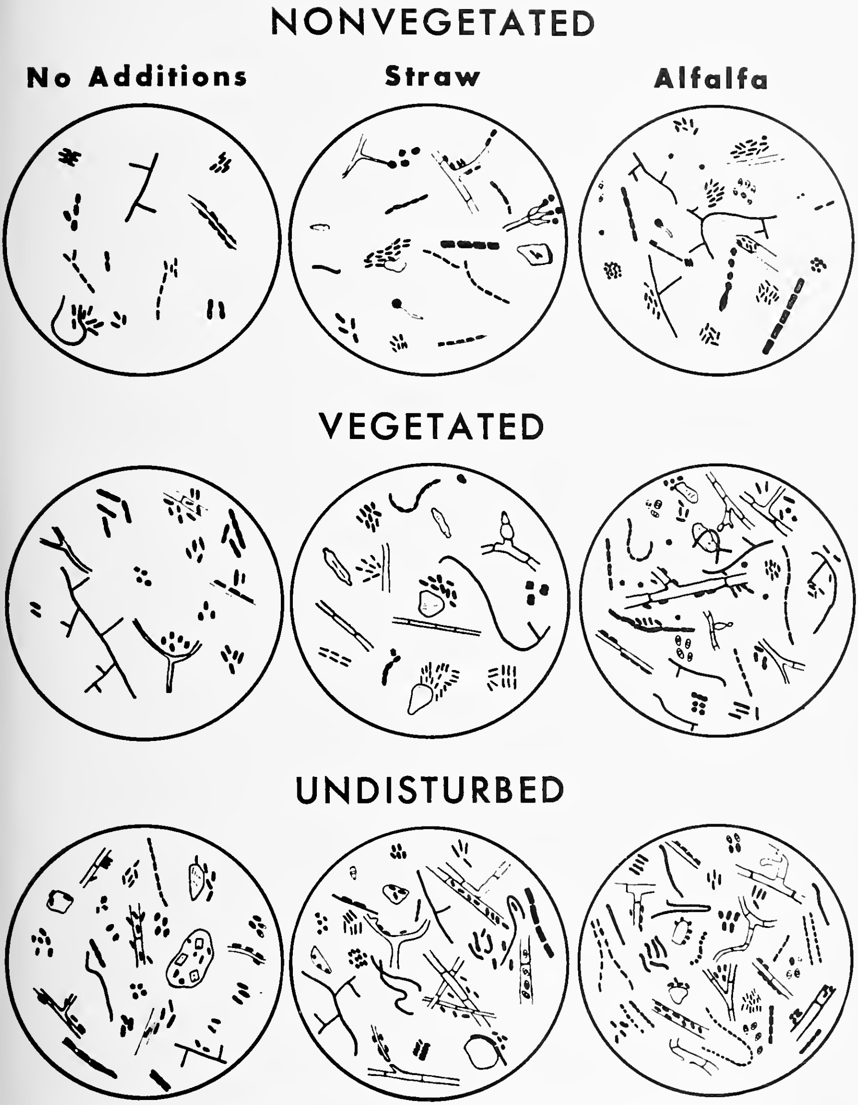

FIGURE 2. Composite microscopic fields drawn from actual observations.

was the absence of relatively large areas of the slides almost devoid of microorganisms as was observed on slides from the spoil. Colonies of bacteria as well as scattered cells, occurring singly or in pairs, were present when straw was added to the soil. Spiral and long spindle-shaped forms were also present. Fungi and actinomycetes were numerous. Larger numbers of microorganisms seemed to result from the addition of alfalfa than from the addition of straw.

NUTRITIONAL REQUIREMENTS (28)

In recent years many workers have been placing more emphasis on the nutritional requirements of soil and rhizosphere isolates than on their taxonomic classification. Taylor (21), in a discussion of water bacteria and their nutritional requirements, stated that little purpose is served in the bacteriology of water by the usual methods of classification. The same might be said of soil bacteria. This study was made to determine the nutritional requirements of some bacterial isolates from spoil.

Methods[4]

The nutritional requirements of 617 isolates from samples obtained from the Bruceton and Pennsylvania areas were determined. The isolates were obtained from plates that had been made for total count determinations or from subsequent plating of the samples made solely for the isolation of bacteria.

The nutritional requirements were determined by the method proposed by Lochhead and Chase (15). The media were as follows: B = inorganic salts + glucose medium; A = B + amino acids; G = B + growth factors; AG = B + amino acids + growth factors; Y = B + yeast extract; S = B + soil extract; and YS = B + yeast extract + soil extract. The best growth response, as indicated by turbidity, was assigned a value of 4, and the growth in the other six media were rated by comparison by giving values of 3, 2, 1, or 0. A difference of at least two on the scale was considered to be significant. For example, ratings of 4 and 2 would be considered significantly different but not 4 and 3.

Results and Discussion

The detailed data are presented in Table 4. Lochhead and Thexton (16) stated that among the bacterial isolates from the rhizosphere the largest group are those requiring amino acids for maximum growth (medium A). In this study 62 per cent of the isolates belonged to the group that required yeast extract in the medium; 10.7 per cent required yeast extract and soil extract, and 7.6 per cent grew in the basal medium. Of the 617 isolates from the spoil, 92.4 per cent required "preformed growth factors."

POLYSACCHARIDE PRODUCTION (28)

The formation of soil aggregates results either from the direct or indirect action of bacteria, fungi, and actinomycetes. Workers do not agree, however. Some believe that the mycelial strands and microbial cells hold

[4]Complete details are given in Wilson, *loc. cit.*

Table 4. Nutritional Groups of Spoil Bacterial Isolates

Spoil	B	A	G	AG	Y	S	YS	NG*	Total Number of Isolates
				Per cent of total					
Pennsylvania									
Nonvegetated	0	2	0	0	98	0	0	0	42
Andropogon									
Roots	6	1	18	2	47	0	12	12	49
Rhizoplane	0	8	3	32	47	3	5	3	38
Rhizosphere	0	0	0	2	86	0	11	0	42
Bruceton									
Nonvegetated	6	4	8	2	73	0	6	0	48
Alsike									
Roots	6	8	0	2	64	0	20	4	50
Rhizoplane	10	6	2	0	69	4	8	0	49
Rhizosphere	6	14	0	6	66	0	9	0	35
Alfalfa									
Roots	8	6	2	0	71	0	12	0	48
Rhizoplane	20	28	8	12	4	0	28	0	50
Rhizosphere	9	11	3	0	63	0	14	0	35
Red Clover									
Roots	32	2	41	9	7	0	9	0	44
Rhizoplane	0	0	2	2	88	0	7	0	43
Rhizosphere	4	0	0	0	93	0	4	0	44
Per cent of total	7.6	6.6	6.5	4.7	62	1.1	10.7	3.1	617†

*No growth in any medium.
†Total number of isolates.

the soil particles together, whereas others believe that the particles are cemented together by microbial by-products (13). Polysaccharides constitute one of the by-products that has been studied.

Methods[5]

The polysaccharide-producing ability of bacterial isolates from samples of nonvegetated and vegetated spoil from the Pennsylvania and Bruceton areas was determined. A medium with glucose as the carbon source (14) was used to determine the polysaccharide production. The flasks were incubated for 5 days at 30° C on a reciprocal shaker after which the polysaccharide was precipitated, recovered, dried, and weighed. Production was rated as good, medium, poor, and none, but these were combined into "moderate to good" and "poor to none" in the tables.

Results and Discussion

The results are presented in Tables 5 and 6. There were few polysaccharide producers among the isolates from nonvegetated spoil. In fact, of 87 isolates, only 3 were classed as moderate to good producers. Whether this reflects any inability of polysaccharide producers to sur-

TABLE 5. NUMBER OF ISOLATES CAPABLE OF PRODUCING A POLYSACCHARIDE FROM GLUCOSE AS A CARBON SOURCE

Origin of Isolates	Relative Amounts of Polysaccharide Produced		Total Number of Isolates
	Moderate to Good Production	Poor to No Production	
PENNSYLVANIA			
Nonvegetated	3	35	38
Andropogon			
Roots	12	10	22
Rhizoplane	11	17	28
Rhizosphere	14	20	34
BRUCETON			
Nonvegetated	0	49	49
Alsike			
Roots	30	15	45
Rhizoplane	6	31	37
Rhizosphere	4	32	36
Red Clover			
Roots	27	16	43
Rhizoplane	14	25	39
Rhizosphere	3	41	44
Alfalfa			
Roots	14	26	40
Rhizoplane	2	47	49
Rhizosphere	22	5	27

[5]Complete details are in Wilson, *loc. cit.*

Table 6. Percentage of Isolates from the Various Sources Producing Polysaccharides

Origin of Isolates	Isolates Producing Medium to Good Polysaccharide Production
	Per Cent
Nonvegetated	3.5
Grass	
Roots	54.5
Rhizoplane	39.4
Rhizosphere	41.0
Legumes	
Roots	45.4
Rhizoplane	17.3
Rhizosphere	27.0

vive the unfavorable conditions existing in such spoil is unknown. The data also indicate that less than 50 per cent of the spoil bacteria are good polysaccharide producers.

Chemical Activities of Spoil Microorganisms

MOST PROBABLE NUMBERS (MPN) (33)

Microbial activity in a spoil is responsible for the transformation of certain elements from one form to another. Among these changes are sulfur oxidation, ammonification, nitrification, and cellulose decomposition. Some bacteria involved in such processes are limited by the pH of their environment.

Methods[6]

Two spoil areas, Canyon and Arthurdale, were chosen to determine the MPN of ammonifiers, denitrifiers, nitrifiers, cellulose decomposers, sulfur oxidizers, and nonsymbiotic aerobic nitrogen fixers (*Azotobacter* sp.). The spoil samples represented the 0 to 4-inch depth.

Results and Discussion

The data are presented in Table 7. What portion of the increase in MPN of certain groups of bacteria is the result of vegetation on the spoil and how much results from a more favorable pH cannot be ascertained from these data. On the other hand, it is also true that the more acid a spoil is the more unfavorable it is for the establishment of higher plants and for their continued growth. In such circumstances the pH and vegetation are completely interrelated in their influence upon the numbers of certain bacterial groups. An exception to the foregoing statement

[6]Details are available in Wilson and Stewart, *loc. cit.*

TABLE 7. NUMBER (MPN)* OF CERTAIN PHYSIOLOGICAL GROUPS OF BACTERIA PER GRAM (OVEN-DRY BASIS) IN TWO STRIP-MINE SPOILS

Spoil Areas and Type	Ammonifiers	Denitrifiers	Nitrifiers	Cellulose-Decomposers	Sulfur-Oxidizers	Azotobacter	pH
CANYON							
Nonvegetated	1,500	7,450	0	15	81,050	0	3.25
Vegetated	58,450	62,700	0	750	6,900	0	4.04
Undisturbed	79,950	259,000	55	2,455	10	0	5.40
ARTHURDALE							
Nonvegetated	150	300	0	0	5,450	0	3.84
Vegetated	306,000	465,500	1,350	58,100	52,400	0	6.09
Pine	1,350	311,500	0	10	300	0	4.74
Locust	185,500	246,500	48	200	150	0	4.77
Undisturbed	79,200	814,500	2,000	6,000	15	0	6.00

*Numbers rounded to nearest 5 if less than 100; to nearest 50 if greater than 100.

in spoil are the sulfur oxidizers. These autotrophs obtain their energy from sulfur oxidation and are not influenced by the organic matter. Except for the Arthurdale vegetated area (forage grasses and legumes), all other spoils, regardless of the type of vegetation, have fewer sulfur oxidizers than the nonvegetated spoils. Neither are these bacteria limited by the low pH prevailing in acid spoils since they are actually responsible for the condition. Azotobacter apparently were entirely lacking in all the samples because of their intolerance of acid conditions.

AMMONIFICATION AND NITRIFICATION (32)

Ammonification and nitrification in the soil are biological processes. Both are necessary in transforming the nitrogen of organic materials to ammonium, and then to the nitrite, and finally to the nitrate form. Knowledge concerning these processes in spoil is limited.

The ammonification process is brought about by several groups of microorganisms. According to Waksman and Starkey (26), many fungi and actinomycetes, as well as numerous aerobic and anaerobic bacteria, are capable of liberating ammonium nitrogen from various organic nitrogenous compounds.

Cornfield (5) reported that the accumulation of ammonium nitrogen was generally high in acid and low in neutral and alkaline soil. From a study of some Connecticut soils having pH values of 5.30 to 5.50, Dorsey (8) found that when $CaCO_3$ was added the ammonifying power of the soil organisms increased.

Nitrification, the oxidation of ammonium nitrogen to nitrite and nitrate, is a function of a few autotrophic bacteria. Consequently, the process is limited by some conditions which would have little or no effect upon the process of ammonification.

Methods[7]

(Ammonification.) One hundred-gram (oven-dried basis) portions of Canyon spoil were weighed into each of several 250-ml beakers. The following studies were made: (a) ammonification of different organic nitrogenous compounds (100 mg organic nitrogen per 100 mg spoil), with and without added $Ca(OH)_2$, and incubated for 7 days, and (b) the ammonification rate of spoil to which was added peptone, urea, or egg albumin, incubated for 28 days.

All samples were maintained at 45 per cent of the spoil's water-holding capacity by adding water to replace that lost by evaporation.

[7]Complete details are in: H. A. Wilson and Gwendolyn Stewart, *Ammonification and Nitrification in a Strip-Mine Spoil,* W. Va. Agr. Expt. Sta. Bull. 379T, June, 1955. 16 pp.

The beakers were incubated at 25 ± 1° C. The 28-day samples were thoroughly stirred once each week. The amount of $Ca(OH)_2$ added was the calculated amount, as obtained from buffer curves, to neutralize acidity.

At the end of the incubation period the ammonia nitrogen was extracted from the sample with 2N KCl (acidified with HCl to pH 1.5) and the amount of nitrogen determined by the MgO method. All data are reported as ammonia nitrogen in terms of milligrams of nitrogen per 100 gm of spoil (oven-dried basis).

Results

(Ammonification.) The data are presented graphically in Figure 3. In the nonvegetated spoil (pH 3.29), asparagine was ammonified more readily than any other nitrogenous source tested and egg albumin the least. In the vegetated spoil (pH 3.95) and undisturbed soil (pH 5.73), urea was ammonified most readily followed by asparagine and peptone. Egg albumin was the least readily ammonified.

The rate of ammonification was followed during a period of 28 days using egg albumin, peptone, and urea (Figure 4). There was considerable variation in the ammonification rate of urea in the spoils and soil. On the other hand, the differences were small in the ammonification rates of peptone and egg albumin after 28 days of incubation. In all cases, however, the rate was slowest in the nonvegetated spoil.

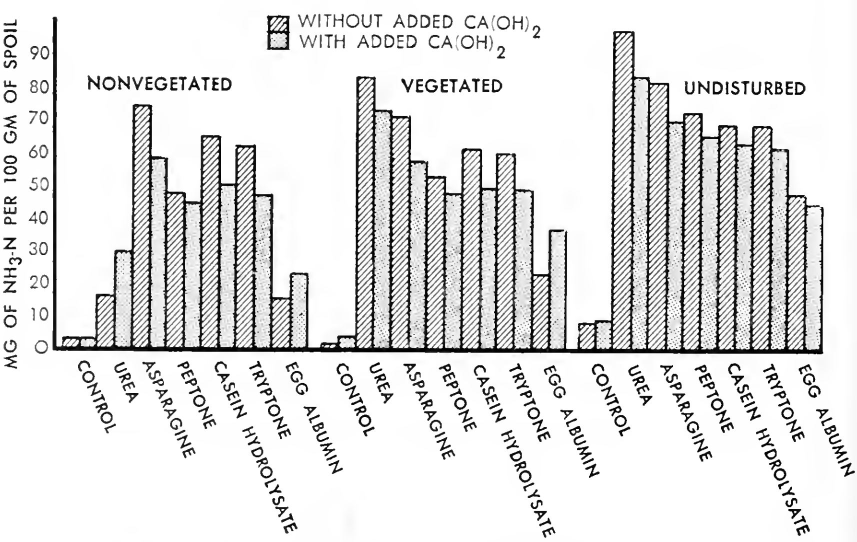

FIGURE 3. Ammonification of various organic nitrogenous materials added to spoil. (Incubated at 25 ± 1° C for seven days.)

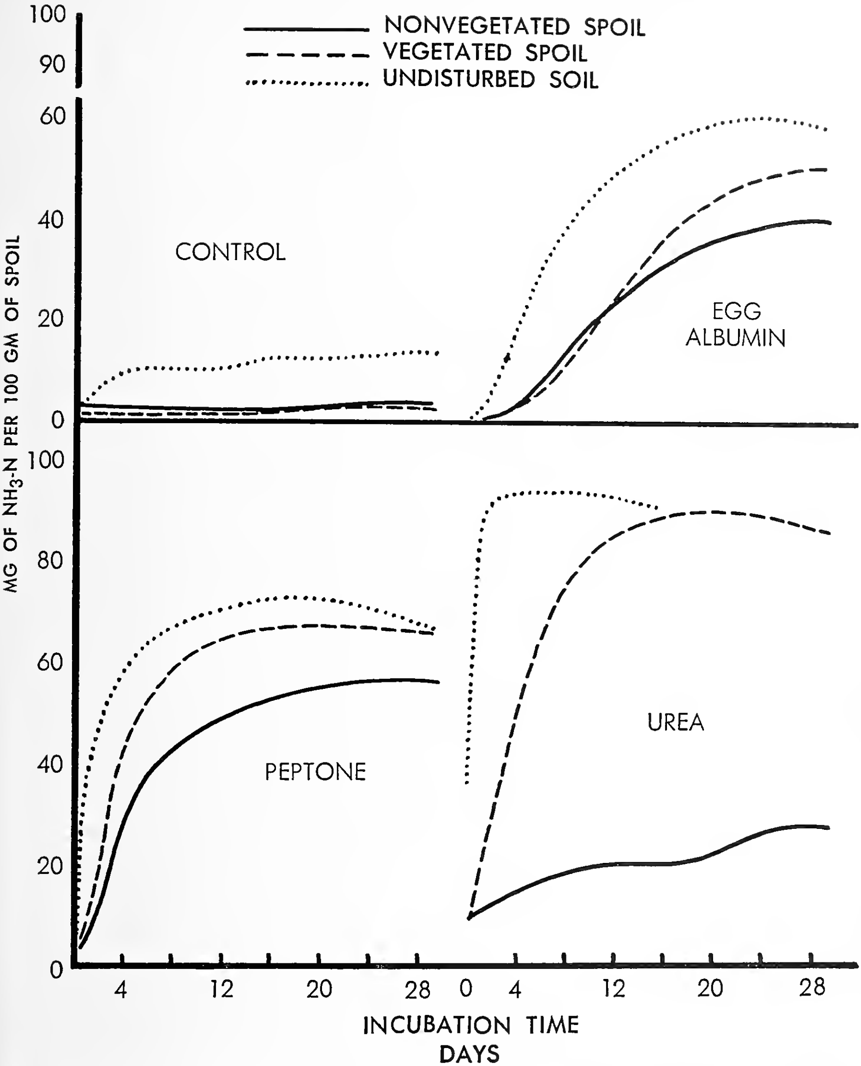

FIGURE 4. The ammonification rate of three organic nitrogen sources added to spoil. (Incubated at 25 ± 1° C.)

Discussion

(Ammonification.) The transformation of organic nitrogen to ammonium nitrogen from different materials incorporated into an acid spoil occurs at varying rates (Figure 3). In the nonvegetated spoil, with a pH of 3.29, the availability of the compounds in terms of ammonium nitrogen accumulated in seven days was in the following decreasing order: asparagine, casein hydrolysate, tryptone, peptone, urea, and egg albumin.

Under vegetated and undisturbed soil conditions more ammonium nitrogen was released from urea after seven days' incubation than from any other material; asparagine was second in yield and egg albumin, last.

In all cases the H-ion concentration decreased as ammonium nitrogen was released from the organic material, and in most cases the sample which contained the largest amount of ammonium nitrogen had the highest pH value.

The addition of $Ca(OH)_2$ to the spoil probably made conditions favorable for an increase in microorganisms, and this may account for the smaller amount of ammonium nitrogen in samples with added $Ca(OH)_2$; apparently some of the ammonium nitrogen had been utilized by the microorganisms.

The nonvegetated spoil appears to be somewhat deficient in those microorganisms capable of transforming the nitrogen of urea to ammonium nitrogen (Figure 4). On the other hand, urea is readily ammonified in the vegetated and undisturbed samples. It seems apparent that a nonvegetated spoil, as acid as pH 3.29, is not a favorable environment for these microorganisms.

The nitrogen of peptone is readily transformed into ammonium nitrogen in the nonvegetated, vegetated, and undisturbed samples; the transformation was slowest with the nonvegetated samples.

These data indicate that the availability of ammonium nitrogen from nitrogenous materials added to spoil will be satisfactory even at the relatively high H-ion concentrations that exist.

Methods[8]

(Nitrification.) The nitrification rate of $(NH_4)_2SO_4$ in spoils was determined with and without the addition of the calculated amount of $Ca(OH)_2$ to neutralize the spoil acidity. The experimental procedure was the same as described under Ammonification. The ammonium nitrogen content of the filtrate was first determined and then the nitrate nitrogen content of the filtrate was determined by the DeVarda's alloy method. The data are reported as nitrate nitrogen in terms of milligrams of nitrogen per 100 gm of spoil on an oven-dry basis.

Results

(Nitrification.) The data are presented in Table 8. The data show that at the low pH levels of the nonvegetated spoil no nitrification occurred within 159 days. On the other hand, the addition of $Ca(OH)_2$ to the spoil did result in some nitrification. Nitrification of the $(NH_4)_2SO_4$ in vegetated spoil did not occur before 97 days of incubation but there

[8] *Ibid.*

Table 8. Nitrification of Ammonium Sulfate Added to Strip-Mine Spoil

Incubation (days)†	Nonvegetated						Vegetated						Undisturbed					
	Without $Ca(OH)_2$			With $Ca(OH)_2$			Without $Ca(OH)_2$			With $Ca(OH)_2$			Without $Ca(OH)_2$			With $Ca(OH)_2$		
	pH	NH_3*	NO_3*	pH	NH_3*	NO_3*	pH	NH_3*	NO_3*	pH	NH_3*	NO_3*	pH	NH_3*	NO_3*	pH	NH_3*	NO_3*
0	3.48	49.47	.55	3.70	47.92	.37	4.13	46.16	.18	4.08	47.26	.18	4.80	49.95	.92	5.03	47.92	.18
5	3.43	50.02	.37	5.95	47.08	.37	4.33	48.55	.37	7.18	46.53	.37	5.55	57.38	.74	6.53	55.35	.37
15	3.38	49.65	.74	6.20	47.74	.37	4.53	47.74	.74	7.13	44.14	.37	5.73	56.24	.74	6.73	57.63	.74
25	3.38	49.65	.55	5.90	45.05	.37	4.55	47.81	.55	6.40	38.99	4.05	5.93	58.85	.55	6.58	56.46	.92
35	3.65	49.10	.55	6.20	44.14	.85	4.70	48.00	.66	5.80	29.42	12.87	6.03	59.22	1.03	6.90	52.96	4.60
41	...	....	..	...	....	....	4.13	46.09	.00	5.18	26.78	16.11	5.45	58.11	.85	5.75	36.49	18.02
47	3.73	46.41	.00	5.98	41.94	1.21	4.43	45.79	.00	4.95	25.75	14.71	5.28	57.56	.29	5.38	37.07	18.76
52	3.48	46.09	.00	5.85	43.33	.18	3.93	46.27	.11	4.53	18.57	21.15	5.35	58.04	.18	4.93	19.68	36.23
57	3.48	47.37	.00	5.73	40.09	1.47	4.35	45.06	.00	4.60	15.01	24.27	5.55	59.88	.66	4.93	5.44	47.37
62	3.58	49.03	.00	5.98	39.91	.37	4.20	45.35	.11	4.53	14.09	23.17	5.30	58.96	.85	4.60	2.94	53.74
67	....	....	..	6.15	41.01	.00	....	....	....	5.25	18.57	20.34	....	....	....	5.10	1.84	54.80
77	....	....	.	....	....	....	....	....	....	4.45	12.32	26.85	....	....	....	4.90	1.54	59.22
97	3.48	48.18	.00	5.35	40.27	2.21	3.90	47.15	.00	....	....	....	4.93	50.68	2.21	....	....	....
159	3.55	45.24	.00	4.58	29.03	11.73	4.40	44.39	2.21	....	....	....	4.23	14.70	46.42	....	....	....

†Samples incubated at 25° ± 1° C.
*Reported in mg of N per 100 gm of spoil (oven-dry basis).

was an accumulation of 2.21 mg of nitrogen as nitrate after 159 days. With the addition of $Ca(OH)_2$, however, the accumulation of nitrates was beginning after 25 days of incubation. The rate of nitrification in the undisturbed soil without $Ca(OH)_2$ was slow but with the addition of the hydroxide the nitrification of $(NH_4)_2SO_4$ was essentially completed after 57 days.

Discussion

(Nitrification.) The nitrification data indicate that without a reduction of the H-ion concentration no oxidation of the ammonium to nitrate nitrogen will take place, particularly in the nonvegetated spoil. Even in the vegetated spoil with a somewhat higher pH, 4.13 as compared to 3.48 in the nonvegetated spoil, nitrification was only beginning at the termination of the experiment, 159 days. In all three samples, with added $Ca(OH)_2$, nitrification does take place, but at different rates. It has been shown that the numbers of nitrifiers are low as determined by the MPN method (see Table 7), even in the undisturbed samples. These data indicate that strongly acid spoils must be limed before the nitrification process will take place. Although such spoils may be supporting vegetation the amount of nitrate formation may be small.

CARBON DIOXIDE EVOLUTION

Carbon dioxide evolution from a soil devoid of vegetation results almost entirely from microbial activity. It, therefore, has been used as an indication of the decomposition of organic matter in soils. Nonvegetated strip-mine spoil may be considered as being devoid of organic matter. Such spoils were shown, however, to have a microbial population (32, 33). Any organic matter added to such spoils would be used as an energy source by the heterotrophic microorganisms present.

A laboratory study was undertaken to determine the amount of carbon dioxide evolved from three spoils to which organic matter was added at the rate of 1 gram per 100 grams of spoil (oven-dried basis). The experimental treatments included the addition of: (a) nitrogen, phosphorus, and potassium (equivalent to 1,000 pounds of a 4-12-4 [N, P_2O_5, K_2O] per acre) either as N, P, K, NP, NK, PK, or NPK, ground wheat straw and $Ca(OH)_2$ (equivalent to neutralize the spoil's acidity) in all possible combinations to nonvegetated, vegetated, and undisturbed Canyon area samples, and (b) nitrogen-phosphorus-potassium (as a complete fertilizer), straw, and $Ca(OH)_2$ in all possible combinations to nonvegetated and vegetated spoil and undisturbed soil from the Dixon and Fairmont areas.

Results[9]

Rate of Carbon Dioxide Evolution (12). (Nonvegetated.) The evolution of carbon dioxide was usually at a maximum, regardless of treatment, on the second, third, or fourth day of incubation. The addition of nitrogen had a greater influence upon the rate of carbon dioxide production than either P or K or PK. This was true even when straw and/or $Ca(OH)_2$ were added. The greatest production of carbon dioxide resulted from the addition of NPK + straw + $Ca(OH)_2$.

(Vegetated.) The control (no additions) had a carbon dioxide production rate essentially equal to the nonvegetated control and the addition of P, K, or PK failed materially to alter the rate. The addition of nitrogen alone, or in combination with other treatments, exerted the greatest influence on the carbon dioxide production. As in the case of nonvegetated spoil, the highest production occurred on the second, third, or fourth day of incubation.

(Undisturbed Soil.) The addition of nitrogen, as in the case of nonvegetated and vegetated spoils, exerted a greater influence upon the rate of carbon dioxide production than any of the other additions. The greatest production followed the addition of NPK + straw + $Ca(OH)_2$, followed closely by NPK + straw, and N + straw + $Ca(OH)_2$.

Results[10]

Amount of Carbon Dioxide Produced (29). Canyon. The amount of carbon dioxide produced in 10 days from all combinations of N, P, K, straw, and $Ca(OH)_2$, when added to nonvegetated and vegetated spoil and undisturbed soil, was determined (Table 9). The nonvegetated spoil (control), and nonvegetated spoil, with added P, K, or PK, evolved essentially equal amounts of carbon dioxide. The addition of N, either with P or K and also in the presence of straw and/or $Ca(OH)_2$, resulted in the greatest evolution of carbon dioxide.

The amount of carbon dioxide produced by vegetated spoil to which N was added, regardless of other additives, was greater than the corresponding treatments with N. The addition of straw and $Ca(OH)_2$ did not result in a significantly greater production of carbon dioxide than the addition of straw alone.

The addition of N to undisturbed soil samples alone, or in combination with any of the other treatments, exerted a greater influence upon the amount of carbon dioxide produced than any other single additive.

[9]Complete details are given in: H. G. Hedrick and H. A. Wilson, "The Rate of Carbon Dioxide Production in a Strip-Mine Spoil," *Proc. W. Va. Acad. Sci.* 28: 11-15. 1956.

[10]Complete details are given in: H. A. Wilson and H. G. Hedrick, "Carbon Dioxide Evolution from Some Strip-Mine Spoils," *Appl. Microbiol.* 5: 17-21. 1957

TABLE 9. CARBON DIOXIDE PRODUCED BY CANYON STRIP-MINE SPOIL TREATED WITH ALL COMBINATIONS OF $Ca(OH)_2$; STRAW; AND NITROGEN, PHOSPHORUS AND POTASSIUM*

TREATMENT†	NONVEGETATED				VEGETATED				UNDISTURBED			
	No $Ca(OH)_2$		$Ca(OH)_2$		No $Ca(OH)_2$		$Ca(OH)_2$		No $Ca(OH)_2$		$Ca(OH)_2$	
	No straw	Straw	No straw	Straw	No straw	Straw	No straw	Straw	No straw	Straw	No straw	Straw
	mg CO_2 per duplicate 100 g of spoil‡											
Control	129	144	135	175	139	154	146	154	436	487	453	545
Nitrogen§	197	267	217	319	234	370	254	339	687	933	793	996
Phosphorus§	124	136	143	211	138	158	140	163	434	492	465	606
Potassium§	119	136	148	223	152	163	150	163	447	503	480	586
Nitrogen + phosphorus	233	265	225	336	230	356	275	356	722	967	809	1064
Nitrogen + potassium	207	289	206	335	235	364	251	372	710	981	733	1094
Phosphorus + potassium	135	145	140	218	154	175	153	155	453	509	474	593
Nitrogen + phosphorus + potassium	214	265	214	383	239	376	242	379	766	981	746	1187
Grand totals				6633				7329				8170

*Incubation at 25° C ± 1 for 10 days.

†$Ca(OH)_2$ for pH adjustment; straw, 1 per cent.

‡Total mg CO_2 produced by duplicate 100 g portions of spoil samples (oven-dried basis).

§Equivalent to 1000 lbs per acre of a 4–12–4 fertilizer.

L.S.D. between grand totals: 5 per cent = 299; 1 per cent = 549. L.S.D. between items in rows and columns: 5 per cent = 35; 1 per cent = 46.

TABLE 10. TOTAL MILLIGRAMS OF CARBON DIOXIDE PRODUCED BY DUPLICATE 100 G PORTIONS (OVEN-DRIED BASIS) OF DIXON STRIP-MINE SPOIL IN 10 DAYS (INCUBATION AT 25° C ± 1)

TREATMENT	NON-VEGETATED	VEGETATED	UNDISTURBED
Control	166	169	193
NPK*	160	178	196
Straw	196	187	236
$Ca(OH)_2$†	155	188	227
NPK + straw	220	206	251
NPK + $Ca(OH)_2$	155	187	219
Straw + $Ca(OH)_2$	213	227	253
NPK + straw + $Ca(OH)_2$	336	244	247
L.S.D. 5%	15	39	n.s.‡
Carbon dioxide produced from all treatments: Total	1501	1586	1822

*NPK = nitrogen-phosphorus-potassium equivalent to a 4–12–4 fertilizer at the rate of 1000 lbs per acre.

†$Ca(OH)_2$ was added to adjust the pH of the spoil.

‡ n.s. = not statistically significant.

L.S.D. between totals: 5 per cent = 54; 1 per cent = 99.

The total amount of carbon dioxide produced during the 10-day period from all treatments by the vegetated spoil was significantly greater than that produced by the nonvegetated spoil and significantly less than that from the undisturbed soil.

Dixon and Fairmont. Only the possible combinations of NPK (as complete fertilizer), straw and $Ca(OH)_2$ as additives to these spoils were studied. The nonvegetated and vegetated samples of the Fairmont area produced amounts of carbon dioxide approximately equal to those produced by the Dixon samples. The results obtained from the Dixon samples are presented in Table 10. The addition of NPK, $Ca(OH)_2$, and NPK + $Ca(OH)_2$ to nonvegetated spoil did not result in an increase of carbon dioxide over the control and the total amount of carbon dioxide evolved from the vegetated spoil by all treatments was significantly less than that from the same treatments to the vegetated spoil. The treatments to the undisturbed soil resulted in a significantly greater production of carbon dioxide than that of the control (no treatment). The total amount of carbon dioxide produced from all treatments of the undisturbed soil was greater than the total amount from all treatments to the vegetated and nonvegetated spoil.

Discussion

Microorganisms capable of decomposing organic matter were present in spoil. These can become an effectual population when conditions are made favorable for their development. Both phosphorus and potas-

sium appeared to be present in sufficient amounts for microbial development. A deficiency of nitrogen and the acidic nature of many spoils are likely to be the limiting factors, the nitrogen deficiency being the more important.

The data indicate that if spoil reaction is adjusted to a favorable level and if nitrogen is added with organic matter (especially organic matter with a wide C/N ratio), then the nitrogen and other elements of the organic matter will be mineralized and become available for the growth of higher plants.

AGGREGATION (27)

The sand, silt, and clay particles in soil become organized into groups called aggregates. The formation of the aggregates is effected by various physical, chemical, and biological factors (1).

Spoil is relatively devoid of aggregates. Yet this material is subjected to the same physical, chemical, and biological forces as soil and its degree of aggregation will be governed only by the limitations imposed by any one factor. Nonvegetated spoil, for example, will develop only limited aggregation. Its organic matter content is almost nil, thereby limiting the activity of saprophytic microorganisms which in turn will limit aggregation.

Methods[11]

Aggregate analyses were made on spoil samples from the Canyon, Kaufmann, Fairmont, Pruntytown, and Arthurdale areas, and the two iron-ore spoil areas designated as Johnson Hollow and Quarry Run.

Twenty-five grams of sieved soil were wet-sieved (34) for 30 minutes on a nest of five sieves. The sieve openings were: 2.00, 1.00, 0.50, 0.25, and 0.10 mm. The material remaining on each sieve was dried and weighed. Microscopic examination of the material remaining on the sieves showed that not all of it was true aggregates. Many of the apparent aggregates were very fine sand or smaller particles of silt and clay that were only coatings for a small piece of rock or coal that did not slake during the wet-sieving process. The actual water stable aggregates therefore were determined by difference. Two samples were weighed. One sample was wet-sieved in the usual manner. The other was first chemically dispersed using sodium metaphosphate (6, 23) as for mechanical analysis, and then wet-sieved. After drying and weighing, the difference between the wet-sieved and the chemically dispersed wet-sieved fractions represented the true water stable aggregate.

[11]Details are given in: H. A. Wilson, "Effect of Vegetation Upon Aggregation in Strip-Mine Spoils," *Soil Sci. America Proc.* 21: 637-640. 1957.

Table 11. Degree of Aggregation of Strip-Mine Spoils, Weight in Grams (Total Weight from Four 25 g Samples)

Spoil Area	Spoil: Untreated	Chemically Dispersed	Degree of Aggregation	L.S.D. 5% Between Degrees of Aggregation
		Coal Spoils		
Canyon				
Nonvegetated	57.2	45.2	12.0	
Vegetated	69.2	45.0	24.2	
Undisturbed	81.3	42.9	38.4	8.6
Kaufmann				
Nonvegetated	50.9	25.0	25.9	
Vegetated	55.7	31.8	23.9	
Undisturbed	82.9	14.8	68.1	5.5
Fairmont				
Nonvegetated	60.3	52.3	8.0	
Vegetated	60.6	43.2	17.4	
Undisturbed	81.2	37.7	43.5	7.2
Pruntytown				
Nonvegetated	64.6	35.4	29.2	
Vegetated	63.6	40.2	23.4	
Undisturbed	77.5	31.9	45.6	3.2
Arthurdale				
Nonvegetated	65.6	47.5	18.1	
Vegetated	80.4	42.0	38.4	
Pine	72.5	44.2	28.3	
Locust	69.1	44.0	29.1	
Undisturbed	87.4	16.2	71.2	5.0
		Iron-ore Spoils		
Johnson Hollow				
Reforested	92.1	47.1	45.0	
Undisturbed	87.5	26.1	61.4	4.5
Quarry Run				
Reforested	84.1	24.2	59.9	
Undisturbed	88.5	30.1	58.4	NS

Results and Discussion

The data are presented in Table 11. The nonvegetated spoils of the Canyon, Fairmont, and Arthurdale areas are not as well aggregated as their vegetated counterparts. For some unexplainable reason, the reverse is true with the Kaufmann and Pruntytown areas. No coal land spoil was as well aggregated as its adjacent undisturbed soil. The aggregation of the pine and locust spoil of the Arthurdale area was about equal; both, however, were less well aggregated than the vegetated spoil and undisturbed soil but better aggregated than the nonvegetated spoil.

The reforested Johnson Hollow iron-ore spoil was not as well aggregated as the adjacent undisturbed soil. On the other hand, the reforested Quarry Run and adjacent undisturbed soil were equally aggregated.

Organic Carbon and Nitrogen Content of Spoil Areas

In arable soils the organic matter results from plant and animal residues and includes the microflora and microfauna. The total carbon under such conditions is the total organic carbon unless carbonates are present. If carbonates are present they are determined and subtracted from the total carbon to obtain the total organic carbon. There are small pieces of coal in spoil samples and since coal is primarily carbon this is a source of error in any determination of organic carbon.

Nitrogen is closely associated with organic carbon in soil and consequently in spoil. Coal also contains nitrogen; therefore a determination of total nitrogen in spoil is in error if coal is present.

An attempt was made to determine the amount of coal in spoil samples and to apply a correction to the determination of organic carbon and total nitrogen content of spoil.

Methods

Sixty-three samples (20 areas) were included in this study; six samples represented three iron-ore spoils. In some instances an undisturbed spoil was common to two spoil areas.

The total carbon was determined by the wet-combustion method. Since it is stated that "elemental carbon," as charcoal and coal, is practically unattacked in this method, this source of error should be eliminated (18). However, in these studies, coal separated from some spoils was heavily attacked during the wet combustion, whereas coal from other spoils was only slightly attacked. The total nitrogen was determined by the Kjeldahl-Gunning method.

The percentage of coal in a spoil sample was determined as follows: a 50-gm sample of spoil was placed in a 500-ml Erlenmeyer flask and to it was added 62 ml of a 10 per cent solution of Calgon[12] and 300 ml of distilled water. The flask was stoppered and the contents shaken for 10 minutes on an equipose shaker after which the contents were poured onto a 140-mesh sieve and the material remaining on the sieve was gently washed with tap water. The material was then removed from the sieve and dried at 105-110° C. It was then placed in a tall form 600-ml beaker and approximately 250 ml of carbon tetrachloride was added. Following the settling of the heavier particles, the carbon tetrachloride was decanted through a 140-mesh sieve. The floating coal was retained on the sieve. The treatment with the tetrachloride was repeated twice. The coal was then retrieved and dried at 105-110° C.

[12]Calgon, a trade name of Calgon Co., Pittsburgh, Pa., is sodium meta-phosphate buffered with sodium carbonate.

Results

The content of coal in the nonvegetated spoils ranged from 0 to 11 per cent and from 0.05 to 17.9 per cent in the vegetated spoil. The total organic carbon, corrected for the carbon in the coal present, averaged 0.65 per cent in the nonvegetated spoil, 0.93 per cent in the vegetated spoil, and 2.32 per cent in the undisturbed soil.

The corresponding percentages of total nitrogen in the nonvegetated and vegetated spoils and undisturbed soil, respectively, were: 0.075, 0.110, and 0.245 per cent. These also were corrected for the coal present.

The total organic carbon for the three iron-ore spoils averaged 2.17 per cent and the total nitrogen averaged 0.207 per cent. The corresponding percentages for the undisturbed soil adjacent to the iron-ore areas were 4.41 and 0.374 per cent.

Discussion

Since considerable quantities of organic matter were indicated in the nonvegetated spoil, in some instances even more than in the vegetated spoil, one must carefully evaluate the results. The same must be done with the nitrogen content. The error lies in the procedure used. In order to obtain more accurate values a procedure is needed to separate the coal from the spoil but none of the organic matter. The dry-combustion method of organic carbon determination would need to be made upon the whole spoil and also upon the coal found in the spoil. The difference in the two values, if no carbonates are present, should yield a fairly dependable value for the organic carbon, excluding that of coal, in the spoil. A total nitrogen determination on the whole spoil, and also upon the extracted coal, would be required to obtain a dependable nitrogen content.

If it is assumed that the errors in the data obtained in this study have been equally corrected and if the overall average is used, organic carbon and nitrogen are accumulating in vegetated spoils at a slow rate. Many years will be required before the organic carbon accumulation equals that of the adjacent undisturbed soil. In the iron-ore spoils, even after approximately 100 years, the organic carbon and nitrogen content is only approximately half that of the adjacent soil.

Extractable Sulfates and Iron (31)

Acid spots are a common characteristic of spoils resulting from the strip-mining of coal seams that are associated with pyritic or marcasitic materials. These spots appear as moist areas (Figure 5). Acid spots may range in size from a barely discernible moist area to several feet in diameter.

FIGURE 5. An acid spot on strip-mine spoil.

The formation of the acid spots probably is the result of the oxidation of the sulfides of the iron polysulfide materials to sulfuric acid as described by Colmer and Hinkle (4) in explaining the formation of acid mine water.

Methods

Acid spot samples were obtained from spoil areas within a 50-mile radius of Morgantown, West Virginia. Samples (0 to 3-inch depth) were obtained from spoil acid spot areas representative of the following conditions: (a) acid spots greater than 15 inches in diameter; (b) acid spots less than 12 inches in diameter; and (c) spoils upon which no acid spots were visible.

Three composite samples were obtained from each acid spot greater than 15 inches in diameter. One sample was taken from the center of the spot, and another from the edge of the spot in such a manner to include equal amounts of material from just within and just outside of the moist acid spot area. The third sample was taken at a distance from the edge approximately equal to the diameter of the spot. Samples representing acid spots less than 12 inches in diameter were composited from the

material within the spot area. A sample also was obtained "diameter distance" from the spot. Each spoil upon which no acid spots were found was represented by only one sample.

The samples were air dried and stored in the laboratory until all determinations except pH were made. The pH determinations were made upon the moist spoil. Sulfates were determined by the method of Chesnin and Yien (2), and iron by the method of Dyer and McFarlane (9). All results are reported in parts per million (ppm) per gram of oven-dried spoil.

Results

IRON. Only a part of the data obtained in the study is shown in Tables 12, 13, and 14. Each table includes only the two lowest values, the two values nearest the average, and the two highest values of the determinations made based upon the sulfates (31).

TABLE 12. EXTRACTABLE SULFATES AND IRON IN ACID SPOTS (PPM) (ACID SPOTS GREATER THAN 15 INCHES IN DIAMETER)*

Sample† Number	Center of Spot			
	Sulfates	Ferrous Iron	Total Iron	pH
8	23	1	60	3.50
21	33	T‡	44	3.50
32	150	4	272	3.50
30	170	5	680	3.40
1	265	19	5000	2.40
26	554	37	1392	2.35
Sample† Number	**Edge of Spot**			
	Sulfates	Ferrous Iron	Total Iron	pH
8	45	1	28	3.70
21	48	1	35	3.60
32	86	3	70	3.50
30	160	3	51	3.30
1	74	1	95	3.65
26	114	3	76	2.50
Sample† Number	**Outside of Spot**			
	Sulfates	Ferrous Iron	Total Iron	pH
8	3	T	9	4.25
21	14	T	21	3.80
32	51	0	6	5.10
30	40	1	127	6.85
1	3	1	22	8.00
26	33	T	11	3.20

*The values represent the two lowest, the two nearest the average, and the two highest values based upon the sulfate content of the "center" samples only.

†Six from a total of 25 samples.

‡Trace.

TABLE 13. EXTRACTABLE SULFATES AND IRON IN ACID SPOTS (PPM) (ACID SPOTS LESS THAN 12 INCHES IN DIAMETER)*

SAMPLE† NUMBER	WHOLE SPOT			
	SULFATES	FERROUS IRON	TOTAL IRON	pH
22	38	1	51	3.30
39	41	3	82	4.50
43	170	T‡	95	3.50
42	185	T	76	3.10
35	275	177	680	3.00
44	350	3	253	3.90
SAMPLE† NUMBER	OUTSIDE OF SPOT			
	SULFATES	FERROUS IRON	TOTAL IRON	pH
22	16	1	13	4.00
39	5	1	9	4.20
43	26	1	24	4.00
42	57	1	9	3.70
35	57	T	22	5.70
44	120	1	24	7.60

*The values represent the two lowest, the two nearest the average, and the two highest values based upon the sulfate content of the "center" samples only.

†Six from a total of 13 samples.

‡Trace.

TABLE 14. EXTRACTABLE SULFATES AND IRON FROM SPOIL WITHOUT ACID SPOTS

SAMPLE*† NUMBER	SPOIL WITHOUT ACID SPOTS			
	SULFATES	FERROUS IRON	TOTAL IRON	pH
49	0	0	5	5.00
50	0	1	4	7.90
11	8	T‡	25	3.70
13	8	0	11	5.00
13	13	0	13	3.85
46	23	0	6	2.90

*The values represent the two lowest, the two nearest the average, and the two highest values based upon the sulfate content.

†Six from a total of 10 samples.

‡Trace.

SULFATES. The sulfates found in the samples from the center, from the edge, and from the area outside the spots greater than 15 inches in diameter, ranged from 23 to 554 ppm, 6 to 195 ppm, and 3 to 102 ppm, respectively. The corresponding average ppm of sulfates in each of these sample groups was 156, 82, and 30.

The range in sulfate concentration for samples representing spots less than 12 inches in diameter was 38 to 350 ppm, with an average of 181 ppm. The range and average for samples representing the area out-

side the spots were 5 to 120 ppm and 45 ppm. The samples from spoils with no visible acid spots contained sulfates ranging from 0 to 23 ppm, with an average of 8 ppm.

Only a small amount of the total iron found in these samples was in the ferrous state. Many of the samples contained no ferrous ions, or at most 1 to 3 ppm. The largest amount of ferrous iron found in any sample was 177 ppm, and only two other samples contained more than 20 ppm. Most of the iron in the samples was in the ferric state (total iron—ferrous iron).

The total iron found in samples from acid spots greater than 15 inches in diameter ranged from 44 to 5,000 ppm, with an average of 713 ppm. The ranges of total iron concentrations in the samples obtained from the edges and outside of these spots was 13 to 222 and 5 and 131 ppm, respectively, with corresponding averages of 79 and 25 ppm.

The samples from acid spots less than 12 inches in diameter had a total iron content which ranged from 51 to 2,625 ppm, with an average of 459 ppm. The range for samples representing the area outside these spots was 9 to 25 ppm and the average was approximately 15 ppm. Samples from spoils on which no acid spots were visible contained from 4 to 25 ppm of total iron and an average of 9 ppm, which corresponds closely to those results obtained from samples taken from the area outside the smaller spots.

pH. The pH determined for the samples representing the center of the spots greater than 15 inches in diameter ranged from 2.05 to 3.60, with an average of 3.04. The corresponding values for the samples representing the edge of the spots and those representing the outside area were 2.40 to 3.90 and 3.34 average, and 2.45 to 8.00 and 4.56 the average, respectively. The range of pH of the samples obtained from acid spots less than 12 inches in diameter was from 2.35 to 5.00, with an average of 3.36. The corresponding pH values for the area outside the spots were 3.55 to 7.60, with an average of 5.21. Samples from spoil areas showing no visible acid spots had pH values ranging from 2.90 to 7.90, with an average of 4.62.

Discussion

The data reveal that large quantities of sulfate (potential sulfuric acid) and iron are associated with acid spots which form on certain coal land spoils. It is probable that ferrous sulfate and sulfuric acid are formed during the oxidation of the iron sulfides in acid spots as has been shown to occur in acid mine drainage waters (4). The ferrous ions under these conditions are soon oxidized to ferric ions thereby forming ferric sulfate.

In arable soils the total sulfur content may be more than 1,000 pounds per acre. Most of this sulfur is in organic combination and as such is not considered available to plants. Upon decomposition of the organic matter, however, sulfates soon appear, but, instead of existing as free sulfuric acid as occurs in spoil, they combine with bases present to form salts. Spoils are probably low in bases, therefore, the free sulfuric acid resulting from oxidation of sulfuritic materials, as well as that from the hydrolysis of any ferric sulfate to ferric hydroxide, remains. This acid subsequently dissociates and is responsible for the acidic nature of the spoil.

Ignatieff, according to Lyon and Buckman (17), visualized a dynamic equilibrium between ferrous and ferric iron in the soil, with the ferric iron predominating when good aeration prevails. This probably explains the small quantities of ferrous iron and the much greater quantities of ferric iron (total minus ferrous) found in these samples. The quantity of iron in spoil is perhaps of little importance, except as it is related to the sulfuric acid released as the ferric sulfate is hydrolyzed to ferric hydroxide.

With few exceptions, acid spots and the spoil near them are too acid for successful revegetation. Even spoil without visible acid spots may be so strongly acid as to doom any planting on the spoil unless adequate lime is added to neutralize the existing acidity.

Abandoned Strip-Mine Areas As Sanitary Landfill Sites (7)

Abandoned strip-mine areas are sometimes used by communities for the disposal of household refuse. Some of these areas can, and perhaps should, be used as landfill sites. A study was conducted to determine whether spoil, if used as a covering material in a sanitary landfill, would in any manner reduce the rate of refuse decomposition.

Methods[13]

Samples of Cheat and WV-Pa. spoils were used in this study. The samples represented the 0 to 5-inch depth. The refuse was obtained from home garbage cans, the inert materials removed, and then ground in a hammer mill. Oxygen uptake was used as an indication of microbial activity or decomposition, and it was determined using a modified Haldane respirometer.

[13]Complete details in: Ann L. Dobson and H. A. Wilson, "Refuse Decomposition in Strip-Mine Spoils," *Proc. W. Va. Acad. Sci.* 35: 59-65. 1963.

Results

Nonvegetated and vegetated spoil were used and the results are presented graphically in Figure 6. The endogenous respiration of the nonvegetated spoil was low. Although not shown, that of the vegetated spoil (poverty grass) was practically identical. On the other hand, the daily O_2-uptake by the refuse alone showed an overall gradual increase during the experimental period. The mixing of spoil and refuse resulted in greater O_2-uptake than the refuse alone.

Although considerable variation occurred from one "run" to the next, the data showed the same trend. When andropogon spoil was mixed with the refuse the O_2-uptake was somewhat greater than when nonvegetated spoil was used; both showed a greater O_2-uptake than the refuse alone. The same general trend was obtained when spoil from the WV-Pa. area was used. The total O_2-uptake by the various combinations of refuse and nonvegetated spoil is shown in Figure 7.

Discussion

These data indicate that certain abandoned strip-mine areas could be used by municipalities as sanitary landfill sites. The acid spoil used as a layering material would exert little or no influence upon the decomposition of the refuse. Neither would the barren character of most acid spoils retard the decomposition rate. Actually, once a landfill is started most of the earth material used for layering, although perhaps not as

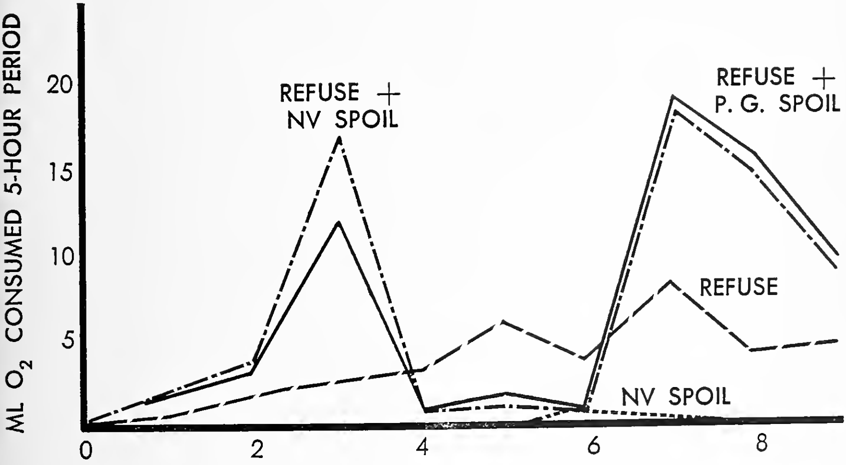

FIGURE 6. Respiration. Household refuse alone and mixed with strip-mine spoil. Nonvegetated spoil and spoil vegetated with poverty grass.

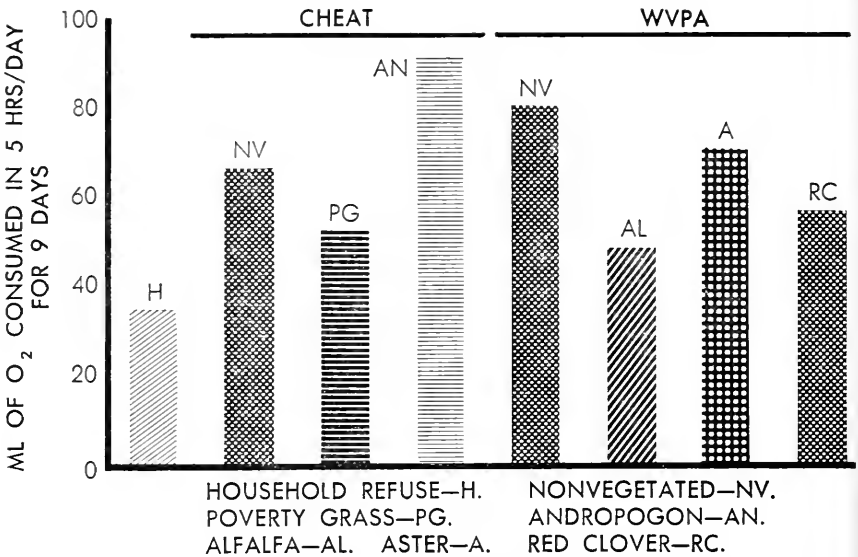

FIGURE 7. Respiration of strip-mine spoil mixed with household refuse.

strongly acid as spoil, is lacking in microbial numbers. Of course, other factors such as location, ease of access, and practicability must be considered.

Summary and Conclusions

The data indicate that strip-mine spoil will not remain a "sterile" area of topography as far as microorganisms are concerned. It may be stated that:

1. The numbers of bacteria, fungi, and actinomycetes will increase in spoils as vegetation becomes established.

2. Vegetation exerts a greater influence upon the spoil microflora than does the pH.

3. Larger numbers of bacteria were found on root surfaces and in the rhizoplane (silt and clay) than in the rhizosphere.

4. Essentially the same fungi were found in nonvegetated spoil and in vegetated spoil.

5. The addition of organic matter to spoil influences both the numbers and types of organisms. An organic material with a narrow C/N ratio (alfalfa) has a greater influence upon the microflora than one with a wide C/N ratio (straw).

6. Most of the isolates from spoil seem to require certain unknown factors in yeast extract in order to grow in a basal medium plus glucose. This indicates some deficiency in the synthetic ability of these isolates.

7. Polysaccharides, a microbial by-product, have been shown to be involved in soil aggregation. Most bacterial isolates from nonvegetated spoil were poor polysaccharide producers. Less than 50 per cent of the vegetated spoil isolates were classed as good polysaccharide producers. The data indicate that good polysaccharide producers may be associated with certain plants.

8. Organisms such as ammonifiers, denitrifiers, nitrifiers, and cellulose decomposers were more numerous in vegetated spoil than in nonvegetated spoil. These organisms, except for the nitrifiers, are heterotrophic and require organic material for energy. The nitrifiers as a group, as well as *Azotobacter* sp., are not especially acid tolerant. The sulfur oxidizers are responsible for the spoil's pH but they do not appear to be influenced by the vegetation.

9. Ammonifying organisms are present in the spoils but considerable variation occurs in the ammonification rate of different materials added to the spoil. The rate was generally a little better in vegetated spoils than in nonvegetated spoils, but in neither was it as good as in undisturbed soil.

10. Nitrification in untreated nonvegetated spoil was nil but the addition of $Ca(OH)_2$ resulted in some nitrification after a 3- to 6-month incubation period.

11. Nitrification in vegetated spoil was extremely slow but following the addition of $Ca(OH)_2$ it became active in about 30 days. The pH of the adjacent undisturbed soil was below 5.0 and nitrification in it was enhanced by the addition of $Ca(OH)_2$. Liming of acid spoils will be necessary before good nitrification can be expected.

12. Organic matter added to strongly acid spoils will decompose but decomposition is enhanced by the addition of nitrogenous materials, particularly if the organic matter has a wide C/N ratio. The addition of lime to neutralize the acidity also results in increased microbial activity. The addition of phosphorus and potassium has little effect upon the decomposition rate.

13. Aggregation is essentially lacking in nonvegetated spoil. If the spoil is vegetated some aggregates are found but the degree of aggregation of the vegetated spoil is much less than that of adjacent undisturbed soil. Under forest conditions nearly 100 years were necessary for iron-ore spoil to become as well aggregated as the adjacent unstripped soil.

14. The accumulation of carbon and nitrogen in vegetated spoils was slow. Furthermore if the spoil contains coal, the usual methods of carbon and nitrogen determinations are not reliable.

15. The oxidation of the iron sulfides to sulfates (sulfuric acid) may be so great as to form acid spots on the spoil surface. These spots may contain large quantities of ferric iron and sulfates, but little ferrous iron. In many instances, a spoil devoid of visible acid spots may contain a considerable amount of sulfates and be strongly acid.

From the data presented, the beneficial influence of vegetation upon the development of the microbial flora is greater than the detrimental influence of pH. The influence of pH upon the vegetation, however, must be recognized. The biochemical activities are similarly influenced. Regardless of the unfavorable environmental conditions of spoil, if vegetation can become established and is able to maintain itself, the microorganisms also will become established. Under such conditions the elements taken up by the plants will become transformed from organic combinations to inorganic forms by microbial action on plant residues.

The liming of the spoils, and proper fertilization, as areas are reseeded to forage crops and legumes will hasten the naturally slow build up of organic matter and nitrogen accumulation in the spoil. Periodic testing of the spoil pH would be desirable. Further additions of lime could be made if the spoil has a tendency to return to a strongly acid condition.

Nature will in time heal these man-made scars but a knowledge of the microbial processes that occur in spoil should enable us to speed up the healing process.

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